OVIPOSITION STRATEGY AND BEHAVIOR OF THE KARNER BLUE BUTTERFLY, *LYCAEIDES MELISSA SAMUELIS* (LYCAENIDAE)

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ABSTRACT. Lepidoptera are known to oviposit on host-plants singly or gregariously, and each particular strategy is thought to aide in larvae survival. We studied the oviposition strategy of the Karner blue butterfly (Lycaenidae), *Lycaeides melissa samuelis* Nabokov, which has two distinct broods per year. We found the Karner blue butterfly oviposition strategy differed between the two broods, but fecundity did not differ. The first brood primarily oviposited singly on host-plants (1.06 eggs/location), whereas the second brood oviposited in clumps (2.94 eggs/location). From these data and noted behavior observations, we hypothesize that the change of strategy is due to environmental conditions and the risk associated with larval aggregation.

Additional key words: host-plant quality, gregarious, larvae, fecundity, risk

The ecology of oviposition behavior in Lepidoptera has been intensively studied from an ecological and evolutionary perspective. Host-plant quality is well known to affect butterfly oviposition preferences and behavior (Grossmueller & Lederhouse 1985; Myers 1985; Singer 2003). Additionally, several studies have focused on the evolutionary causes, benefits, and risks of having gregarious eggs and larvae (Stamp 1980; Courtney 1984; Inouye & Johnson 2005). However, we are not aware of any study which has examined a species that changes oviposition behavior with their particular generation. Data on such behavior changes may provide insights into the trade-offs of ovipositing singly or in larger clumps. Behavior changes may also reveal environmental conditions that correlate with particular oviposition strategies.

The federally endangered Karner blue butterfly (Karner blue) (Lycaenidae), *Lycaeides melissa samuelis* Nabokov, is an inhabitant of oak savanna and pine barrens in the Midwestern and Eastern United States. The Karner blue feeds exclusively on wild blue lupine, *Lupinus perennis* L. (Fabaeeae), always has two broods per year, and has a mean adult lifespan of 3.5 days (Knutson *et al.* 1999). The life cycle of the Karner blue is described in Figure 1. Throughout the Karner blue range, dry conditions are relatively common during the second brood larval phase, and host-plant senescence at this time decreases leaf nitrogen and water in host-plants (Pickens & Root 2008). The result is likely to be increased second brood larvae mortality and/or poor physical condition of adults. In this study, we report the Karner blue oviposition strategy, oviposition behavior, and estimate the fecundity of females for each of the two broods.

MATERIALS AND METHODS

Our study took place at four sites in Lucas County, Ohio, USA and the sites were located at The Nature Conservancy's Kitty Todd Preserve (41° 37' N, 083°47' W). The four sites were separated by distances ranging from 75 to 660 meters. The plant community was a black oak/lupine savanna as described by NatureServe (2006). Dominant woody vegetation included *Quercus velutina*, *Quercus ellipsoidalis*, and *Quercus alba* with a tallgrass prairie herbaceous layer.

We used modified Pollard-Yates transects (Thomas 1983; Pollard & Yates 1993) to survey Karner blues daily for the first and second brood in 2005. One to three trained observers performed Karner blue surveys throughout all host-plant areas at the four sites. When a female Karner blue was observed, we performed a 15-minute behavior observation. During the second brood, 16 of 121 observations were performed for only 10 minutes since a larger population of butterflies was anticipated. If a butterfly was demonstrating obvious oviposition behavior after 15 minutes, we continued the observation for three additional minutes. We recorded oviposition locations, total time of observation, number
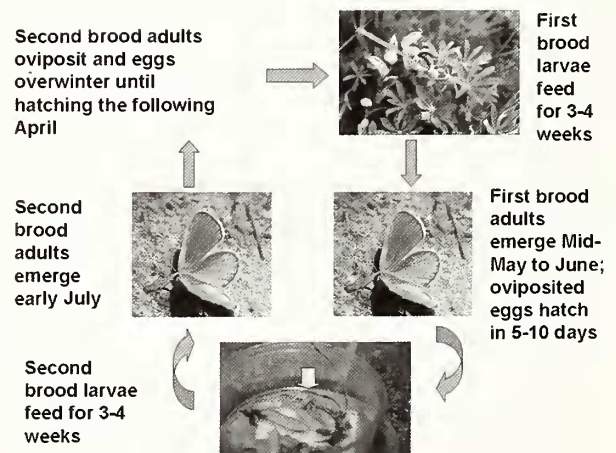

FIG. 1. Life cycle of the Karner blue butterfly, *Lycaeides melissa samuelis*. Photograph of adult male by Marcus Ricci.

of ovipositions, and substrate of oviposition. Since individual *L. perennis* are difficult to distinguish in the field (Grigore & Tramer 1996), a single location was recorded if eggs were within the same 1 m² area. We acknowledge that our definition of clumped differs somewhat from studies where larvae are certain to directly interact with each other. However, plants within 1 m² experienced similar environmental conditions (e.g. shade, moisture) and might have even been the same individual plant.

Two HOBO temperature data loggers (Onset Computer Corporation, Bourne, MA) were placed in an open, sunny area and a well-shaded area at one study site. Temperatures were recorded every 40-minutes and were correlated with survey and oviposition dates and times. SAS 8.01 (SAS Institute 2000) was used for data analysis, and means are reported with ± standard error.

RESULTS

Oviposition Behavior Description. During hot and dry weather periods late in the Karner blue's first brood, females were commonly observed alighting on host-plant leaves and moving in a tight circle while batting their antennae against *L. perennis* leaves. The butterflies were then observed either to crawl down the stem and oviposit or move to another host-plant. For the latter, oviposition often occurred after alighting on 2–5 different *L. perennis*. This circling behavior was much more prevalent late in the first brood compared to other periods, although we did not quantify the exact frequency of the behavior. The circling behavior has previously been observed in a captive setting and is suggested to be a method used by the butterfly to cool itself (Lane 1999). In contrast to Lane's observations, the pattern we observed was inconsistent with heat being the sole factor causing the behavior. The second brood adult period in July was much hotter than the first brood (unpublished temperature logger data), and yet the circling behavior was rarely observed with second brood adults. In contrast, second brood adults went straight to host-plants and selected locations to oviposit without circling on individual leaves. During the first brood of Karner blues, ovipositions were only on lupine stems. For the second brood, we observed Karner blues ovipositing on lupine (*L. perennis*) (79.8%), grasses (16.7%), dewberry (*Rubus villosus*) (1.2%), early goldenrod (*Solidago juncea*) (1.2%), and on the ground (1.2%).

Oviposition Strategy and Rate. We observed 46 ovipositions in 58 observations for the first brood and 84 ovipositions in 122 observations for the second brood. The frequency of oviposition per observation (1 or 0) differed between Karner blue broods. The first brood oviposited at least one egg per observation more often than the second brood (Fisher's exact test, df=1, $\chi^2=10.6$, p<0.002, 45% vs. 21%). However, when Karner blues did oviposit, the mean number of ovipositions per location (within 1m²) for the second brood was greater than the first brood (Wilcoxon 2-sample test, n=51, 2.94 ± 0.30 vs. 1.06 ± 0.04 eggs/location, Z=-5.24, p<0.0001). Essentially, eggs were oviposited individually in the first brood and oviposited in clumps more often during the second brood.

We used Lane's (1999) female Karner blue movement threshold of 24.6°C and assumed Karner blues oviposited at temperatures above this threshold. From the temperature loggers, we estimated the number of hours available for oviposition behavior was 10.7 hours/day for the first brood and 11.3 hours/day for the second brood. Multiplying by the oviposition rate for each brood, we estimated each female oviposited 34.9 eggs/day for the first brood and 34 eggs/day for the second brood. Since Karner blues have a mean adult lifespan of 3.5 days (Knutson *et al.* 1999), we estimated 139.6 eggs/female for the first brood and 136 eggs/female for the second brood.

DISCUSSION

Our study found that Karner blues changed oviposition strategy and behavior based on their generation, which may also correlate with environmental characteristics. Lepidoptera species are known to oviposit using either a clumped strategy or by ovipositing singly, but generally not both strategies. The first brood of Karner blue adults distributed their ovipositions as much as possible by primarily ovipositing eggs singly on host-plants, while the second brood adults oviposited 1–6 eggs per 1 m². Nevertheless, a similar number of eggs were produced for both broods. These results complement previous research, which show Karner blue population growth rates are more density-dependent for the first brood adults (i.e. higher first brood abundances lead to lower per capita successful reproduction) compared to the second brood adults (Pickens 2007). If lupine is a limiting factor, the host-plant should be more limited for the first brood adults because it appears optimal for Karner blues to spread their ovipositions on many lupine plants during the first adult generation.

There are two distinct possibilities for the observed difference in oviposition strategy between broods. First, Karner blues are known to be vulnerable to droughts in June-July (Maxwell 1998; Piekens 2007), so the eggs could be spread as much as possible by the first brood adults to avoid a catastrophe caused by the senescence

of a small group of host-plants. The trade-off involves the disadvantage of expending more time and energy to oviposit and the advantage of optimal oviposition placement. Time for oviposition is crucial because researchers have found butterflies to be more limited by time for oviposition rather than the number of eggs in the oviduct (Courtney 1984; Doak *et al.* 2006). Therefore, we would expect the single oviposition strategy to be advantageous for second brood larvae survivorship. The circling behavior observed could be attributed to butterflies searching for plants with a higher water content and later senescence. Of course, host-plant nitrogen or water content is unlikely to be selected for by second brood adults since their eggs do not hatch until after the winter season.

A second hypothesis for a differing oviposition strategy is that the survivorship of eggs through the nine month overwintering stage could be less than the survival of eggs for the 5–10 day period in summer. For example, three or four feeding larvae could compete for host-plant foliage on the same host-plant, but if only 1 of 4 eggs survive through the winter, there would be no larval competition. Each of the two hypotheses is plausible and a combination of these two theories could also have lead to the observed behavior of the species. Future studies could assist in determining if this behavioral strategy is a response to host-plant conditions, which differs between the two broods.

Our oviposition rate estimates are limited by a one year study period. However, fecundity for Karner blues has only been reported for individuals taken from the wild without knowledge of age or nutritional conditions. Our methodology found fecundity of females in the field (139.6 or 136 eggs per female) to be comparable to the number of eggs produced by females brought into captivity, which has been noted at a maximum of 200 eggs, but is usually closer to 100 eggs (unpublished reports, Toledo Zoo). In conclusion, we have documented oviposition rates of a bivoltine species and we found a shift in Karner blue oviposition strategy between the two broods which may indicate a relative survival advantage for the species.

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